

(E)-5-(2-Bromovinyl)-2'-deoxyuridine: A potent and selective anti-herpes agent

[antiviral activity/antimetabolic activity/(E)-5-(2-iodovinyl)-2'-deoxyuridine/5-iodo-2'-deoxyuridine]

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ABSTRACT Of a series of five newly synthesized 2'-deoxyuridine derivatives, including 5-vinyl-dUrd, 5-ethynyl-dUrd, 5-(1-chlorovinyl)-dUrd, (E)-5-(2-bromovinyl)-dUrd, and (E)-5-(2-iodovinyl)-dUrd, the last two compounds were found to exert a marked inhibitory effect on the replication of herpes simplex virus type 1 [ID₅₀ (mean inhibitory dose), 0.004–0.02 µg/ml]. Both (E)-5-(2-bromovinyl)-dUrd and (E)-5-(2-iodovinyl)-dUrd were highly selective in their anti-herpes activity in that they did not affect the growth or metabolism of the host (primary rabbit kidney) cells unless drug concentrations were used that were 5,000- to 10,000-fold greater than those required to inhibit virus multiplication. In this sense (E)-5-(2-bromovinyl)-dUrd and (E)-5-(2-iodovinyl)-dUrd proved more selective in their activity against herpes simplex virus type 1 than all other anti-herpes compounds that have been described so far. In animal model systems (namely, cutaneous herpes infections of athymic nude mice), (E)-5-(2-bromovinyl)-dUrd suppressed the development of herpetic skin lesions and mortality therewith associated, whether the compound was administered topically or systemically. Under the same conditions, the standard anti-herpes drug 5-iodo-dUrd (Idoxuridine) offered little, if any, protection. Although the precise mechanism of action of (E)-5-(2-bromovinyl)-dUrd and (E)-5-(2-iodovinyl)-dUrd remains to be established, preliminary findings indicate that they do not specifically act at the thymidylate synthetase step.

Idoxuridine (5-iodo-2'-deoxyuridine) and trifluorothymidine (5-trifluoromethyl-2'-deoxyuridine) are among the best known antiviral agents currently used in the chemotherapy of herpesvirus infections (1). Their clinical usefulness is restricted to the topical treatment of local herpes simplex virus (HSV) infections such as herpetic keratitis and "cold sores" (herpes labialis). The unfavorable therapeutic index of Idoxuridine when administered parenterally—as most dramatically exemplified by its inefficacy in patients with herpes simplex encephalitis (2)—has prompted the search for more effective and less toxic anti-herpes agents. In recent years several compounds have been described which are all endowed with selective anti-herpes properties. These compounds include phosphonoacetic acid (3, 4), phosphonoformic acid (5, 6), 9-β-D-arabinofuranosyladenine (araA) (7, 8), 5-iodo-5'-amino-2',5'-dideoxyuridine (AIDDU) (9, 10), 9-(2-hydroxyethoxymethyl)guanine (acycloguanosine) (11, 12), 1-β-D-arabinofuranosylthymine (araT) (13, 14), 5-iodo- and 5-bromo-2'-deoxycytidine (15, 16), 5,6-dihydro-5-azathymidine (17, 18), and erythro-9-[3-(2-hydroxynonyl)]adenine (EHNA) (19). Among the 5-substituted 2'-deoxyuridines, various compounds—namely, 5-methyl-amino-dUrd (20), 5-methoxymethyl-dUrd (21), 5-propyl-dUrd (22), and 5-propynyloxy-dUrd (23)—proved more inhibitory to herpes than to any other DNA (or RNA) virus. We now report on the synthesis and antiviral potency of two new anti-herpes compounds, (E)-5-(2-bromovinyl)-dUrd and (E)-5-(2-iodo-

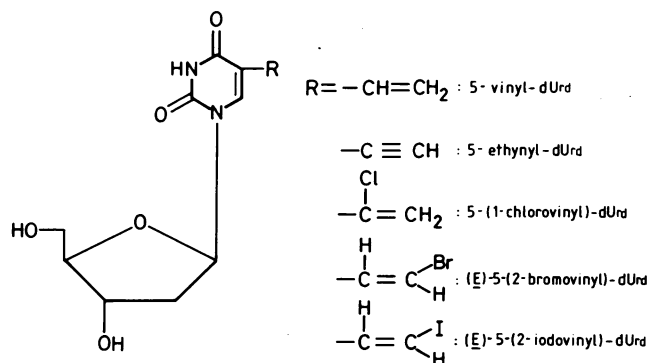


FIG. 1. 5-Vinyl-2'-deoxyuridine derivatives.

vinyl)-dUrd. These compounds surpassed all previously described anti-herpes agents, including acycloguanosine, in both potency and selectivity, at least in primary rabbit kidney (PRK) cells infected with herpes simplex virus type 1 (HSV-1).

Compounds

5-Vinyl-dUrd, 5-ethynyl-dUrd, (E)-5-(2-bromovinyl)-dUrd, and (E)-5-(2-iodovinyl)-dUrd were obtained by condensing the trimethylsilyl derivatives of the appropriate 5-substituted uracils with 2'-deoxy-3,5-di-O-p-toluoyl-α-D-erythropentofuranosyl chloride to give a mixture of the α- and β-anomers of the protected nucleosides. T anomers were separated by chromatography on silica gel and the p-toluoyl protecting groups were removed by treatment with sodium methoxide in methanol. The results indicated for the biological experiments were all obtained by using the β-anomers (Fig. 1). The α-anomers were also tested for biological activity, but they were inactive. The synthesis of 5-ethynyl-dUrd and 5-vinyl-dUrd has been published (24, 25). The synthesis of (E)-5-(2-bromovinyl)-dUrd, (E)-5-(2-iodovinyl)-dUrd, and 5-(1-chlorovinyl)-dUrd has been published elsewhere (ref. 26 and unpublished data). The structures assigned to these compounds were established by elemental analysis and UV and NMR spectroscopy.

The sources of the "standard" anti-herpes compounds were as follows: 5-iodo-dUrd, Ludco (Brussels); 5-trifluoromethyl-dUrd, Sigma; phosphonoacetic acid, Abbott; acycloguanosine, Wellcome (Beckenham, England; courtesy of G. B. Elion); araA, Parke, Davis (courtesy of R. Wolf); araC (1-β-D-arabinofuranosylcytosine), Upjohn; araT, Terra-Marine Bioresearch (La Jolla, CA); 5-ethyl-dUrd, see refs. 27 and 28; 5-propyl-dUrd, see ref. 22; 5-propynyloxy-dUrd, see ref. 23; 5-bromo-dUrd, Sigma; AIDDU, Calbiochem; EHNA, Burroughs Wellcome (Research Triangle Park, NC).

Abbreviations: PRK cells, primary rabbit kidney cells; HSF, human skin fibroblast; PFU, plaque-forming units; HSV-1, herpes simplex virus type 1; araA, 9-β-D-arabinofuranosyladenine; araC, 1-β-D-arabinofuranosylcytosine; araT, 1-β-D-arabinofuranosylthymine; AIDDU, 5-iodo-5'-amino-2',5'-dideoxyuridine; EHNA, erythro-9-[3-(2-hydroxynonyl)]adenine; ID₅₀, mean infective dose.

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Table 1. Antiviral and antimetabolic activities of (*E*)-5-(2-bromovinyl)-dUrd and related 5-vinyl-dUrd derivatives in PRK and HSF cell cultures*

Compound	HSV-1 (KOS)		HSV-1 (HSF)		[methyl- ³ H]-dThd (PRK)	[2- ¹⁴ C]dUrd (PRK)	PRK cell counts	Anti-viral index†
	(PRK)	(PRK)	(KOS)	(HSF)				
5-Vinyl-dUrd	0.04	0.4	0.07	0.2	20	10	8	200
5-Ethynyl-dUrd	0.1	0.2	0.2	0.1	5	0.1	0.3	3
5-(1-Chlorovinyl)-dUrd	0.4	0.7	2	1	25	0.5	2	5
(<i>E</i>)-5-(2-Bromovinyl)-dUrd	0.007	7	0.01	0.7	70	70	>33	>5000
(<i>E</i>)-5-(2-Iodovinyl)-dUrd	0.01	10	0.02	1	70	70	ND	ND
5-Iodo-dUrd	0.2	0.2	0.4	0.2	2.5	1.2	6	30

* Values are in ID₅₀ (μg/ml)—concentration required to reduce virus-induced cytopathogenicity, or [methyl-³H]dThd or [2-¹⁴C]dUrd incorporation into DNA, or total (PRK) cell number by 50%. For measuring the effects of the compounds of viral cytopathogenicity, confluent monolayer cultures (in Linbro microtiter trays) were inoculated with 100 CCID₅₀ (100 × virus dose that infects 50% of the cell cultures) of HSV-1 (KOS) or vaccinia virus and the compounds were added to the cell cultures immediately after virus adsorption. Viral cytopathogenicity was recorded as soon as it reached 100% in the control (virus-infected but untreated) cell cultures. The incorporation of [methyl-³H]dThd and [2-¹⁴C]dUrd into PRK cell DNA was monitored as described (29). PRK cells were seeded in Linbro microwells in the presence of either [methyl-³H]dThd (0.12 μCi/0.01 nmol per 10⁵ cells) or [2-¹⁴C]Urd (14 μCi/250 nmol per 10⁵ cells) and various concentrations of the compounds and allowed to proliferate for 16 hr at 37°C in a humidified, CO₂-controlled atmosphere. PRK cell counts were determined (by Coulter Counter) 3 days after the cells (seeded at 10⁶ cells per petri dish) had been incubated (at 37°C, CO₂) with various concentrations of the compounds. ND, not determined.

† Determined by dividing the ID₅₀ for PRK cell counts by the ID₅₀ for HSV-1 (KOS) replication in PRK cell cultures.

Antiviral activity

Antiviral tests were based on the inhibition of cytopathogenicity induced by either vaccinia or HSV-1 (strain KOS) in PRK and human skin fibroblast (HSF) cell cultures (Table 1). In accord with previous data (30), 5-vinyl-dUrd proved to be more inhibitory to herpes simplex than the standard anti-herpes compound 5-iodo-dUrd (Table 1). Substitution of a chlorine at C-1 of the vinyl group weakened the anti-herpes potency of 5-vinyl-dUrd, whereas substitution of a bromine or iodine at C-2 further increased its anti-herpes activity; the resulting (*E*)-5-(2-bromovinyl)-dUrd and (*E*)-5-(2-iodovinyl)-dUrd inhibited the replication of HSV-1 (KOS) (Table 1) and of various other HSV-1 strains (including clinical isolates) at a concentration of 0.004–0.02 μg/ml. Unlike 5-ethynyl-dUrd and 5-(1-chloro-

vinyl)-dUrd, which inhibited herpes simplex and vaccinia virus to approximately the same extent, (*E*)-5-(2-bromovinyl)-dUrd and (*E*)-5-(2-iodovinyl)-dUrd inhibited HSV-1 (KOS) at a concentration that was lower by several orders of magnitude than the concentration required to inhibit vaccinia virus replication (Table 1). Our recent findings suggest that strains of HSV type 2 are also sensitive to the inhibitory effects of (*E*)-5-(2-bromovinyl)-dUrd and (*E*)-5-(2-iodovinyl)-dUrd but less so than HSV-1 strains.

That the inhibitory effects of the 5-vinyl-dUrd analogues on viral cytopathogenicity truly reflected an inhibition of virus multiplication was confirmed by measuring virus growth in the presence of the compounds. When added to PRK cells at 0.1 μg/ml immediately after the cells had been inoculated with

Table 2. Potency and selectivity of anti-herpes compounds in PRK cell cultures

Compound	Minimum effective dose,* μg/ml (A)	Minimum toxic dose, μg/ml		Antiviral index	
		(B)†	(C)‡	B/A	C/A
Phosphonoacetate	10 (7–20)	80	200	8	20
Acycloguanosine	0.04 (0.02–0.07)	7	50	175	1250
AraA	5 (4–10)	20	80	4	16
AraC	0.04	0.05	0.2	1.2	5
AraT	0.5 (0.1–0.7)	300	≥300	600	≥600
5-Iodo-dUrd	0.15 (0.1–0.2)	1.2	4 [§]	8	27
5-Trifluoromethyl-dUrd	0.6 (0.2–1)	0.05	1 [§]	0.08	1.7
5-Ethyl-dUrd	0.7 (0.4–1)	30	100 [§]	43	143
5-Propyl-dUrd	0.8 (0.2–1)	300	>300 [§]	375	>375
5-Propynyloxy-dUrd	1 (0.4–2)	50	>100 [§]	50	>100
5-Bromo-dCrd	0.1 (0.07–0.2)	50	ND	500	—
AIDDU	25 (10–80)	25	>100	1	>4
EHNA	100	ND	100	—	1
(<i>E</i>)-5-(2-Bromovinyl)-dUrd	0.007 (0.004–0.02)	70	>50	10,000	>7000
(<i>E</i>)-5-(2-Iodovinyl)-dUrd	0.01 (0.004–0.04)	70	ND	7,000	—

* Concentration required to induce cytopathogenicity of three selected HSV-1 strains (KOS, F, and McIntyre) by 50%; the data represent mean values for about 10 separate assays (the range of individual values is indicated in parentheses).

† Concentration required to reduce [2-¹⁴C]dUrd incorporation into host cell DNA by 50%.

‡ Concentration required to reduce cell counts (after 3 days of exponential cell growth) by 30%.

§ As reported previously (31).

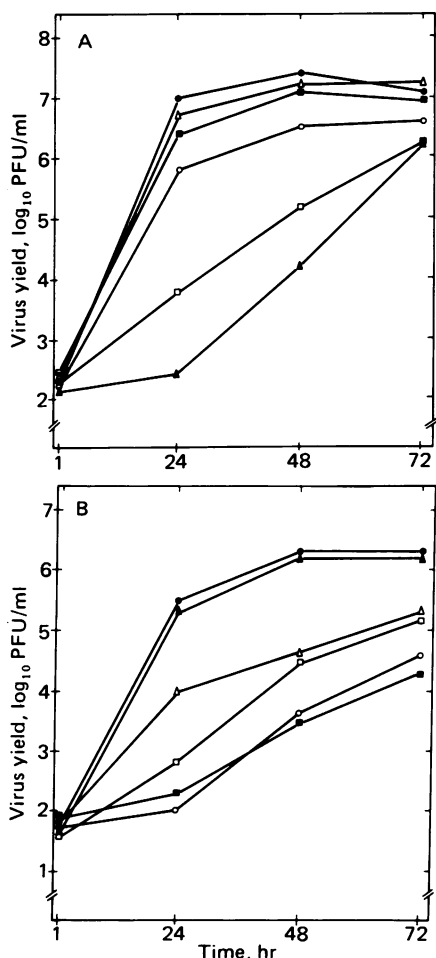


FIG. 2. Effect of 5-vinyl-dUrd analogues on the multiplication of either HSV-1 (KOS) (A) or vaccinia virus (B) in PRK cell cultures. Virus input: 4.5 log₁₀ PFU per petri dish. The compounds were added immediately after virus adsorption, at either 0.1 μg/ml (A) or 1 μg/ml (B). Virus yield was determined by plaque formation in VERO cell cultures [for HSV-1 (KOS)] or PRK cell cultures [for vaccinia virus]. ●, Control; Δ, 5-(1-chlorovinyl)-dUrd; ■, 5-ethynyl-dUrd; ○, 5-iodo-dUrd; □, 5-vinyl-dUrd; ▲, (E)-5-(2-bromovinyl)-dUrd.

HSV-1 (KOS) [0.03 plaque-forming unit (PFU) per cell], only 5-vinyl-dUrd and (E)-5-(2-bromovinyl)-dUrd effected a significant reduction in virus yield (Fig. 2A). (E)-5-(2-iodovinyl)-dUrd was not included in this comparative study. (E)-5-(2-Bromovinyl)-dUrd remained essentially without effect on vaccinia virus growth, even when added to the cell cultures at 1 μg/ml (Fig. 2B). Under the same conditions, 5-(1-chlorovinyl)-dUrd, 5-vinyl-dUrd, 5-ethynyl-dUrd, and 5-iodo-dUrd appreciably reduced the vaccinia virus yield (Fig. 2B). The fact that (E)-5-(2-bromovinyl)-dUrd, while very effective in inhibiting HSV-1 growth, did not markedly affect vaccinia virus multiplication further points to its selectivity as an anti-herpes agent.

Antimetabolic activity

Nucleoside analogues that block dUrd incorporation into DNA at doses that are significantly lower than those required to inhibit dThd incorporation may be assumed to act specifically at the thymidylate synthetase level (29). Indeed, the conversion of dUMP to dTMP is the only metabolic step that distinguishes the incorporation of dUrd and dThd into DNA dTMP. Accordingly, 5-ethynyl-dUrd and 5-(1-chlorovinyl)-dUrd, which suppressed dUrd incorporation into PRK cell DNA at concentrations that were not inhibitory for dThd incorporation (Table 1), could be considered as selective thymidylate synthetase

inhibitors. In this sense, they behaved like 5-fluoro-dUrd, 5-trifluoromethyl-dUrd, 5-nitro-dUrd, 5-cyano-dUrd, and 5-thiocyano-dUrd, which have also been recognized as selective thymidylate synthetase blockers (29). The other 5-vinyl-dUrd analogues, (E)-5-(2-bromovinyl)-dUrd and (E)-5-(2-iodovinyl)-dUrd, and 5-vinyl-dUrd itself inhibited dUrd and dThd incorporation at nearly identical concentrations (Table 1). These nucleoside analogues may be postulated to interfere with one (or more) stages of DNA biosynthesis other than the dTMP synthetase reaction.

Neither (E)-5-(2-bromovinyl)-dUrd nor (E)-5-(2-iodovinyl)-dUrd had an adverse effect on normal cell metabolism (as monitored by either dUrd or dThd incorporation into PRK cell DNA), unless a fairly high concentration (70 μg/ml) was used—that is, a 7000- to 10,000-fold higher concentration than that required to inhibit the replication of HSV-1 (KOS) (Table 1). Little, if any, selectivity was noted in the anti-herpes activity of 5-ethynyl-dUrd and 5-(1-chlorovinyl)-dUrd; these compounds inhibited normal cell metabolism (dUrd incorporation) at concentrations that coincided quite well with the antiviral doses (Table 1). That (E)-5-(2-bromovinyl)-dUrd could be considered as a highly selective anti-herpes agent was further attested by its failure to suppress normal (PRK) cell proliferation. Antiviral indexes, based on the ratio of the ID₅₀ for total PRK cell counts to the ID₅₀ for HSV-1 (KOS) replication, are listed in Table 1. The anti-herpes index of (E)-5-(2-bromovinyl)-dUrd greatly exceeded those of 5-iodo-dUrd, 5-(1-chlorovinyl)-dUrd, 5-ethynyl-dUrd, and 5-vinyl-dUrd (Table 1).

Relative potency and selectivity

Because (E)-5-(2-bromovinyl)-dUrd and (E)-5-(2-iodovinyl)-dUrd appeared to be the most active and selective anti-herpes agents of the 5-vinyl-dUrd analogues (Table 1), their relative potency and selectivity were compared to a wider variety of compounds with established anti-herpes potentials (see introduction). The antiviral activity was assessed with three different HSV-1 strains (KOS, F, and McIntyre), and both inhibition of dUrd incorporation into DNA and inhibition of cell growth were chosen as measures of cytotoxicity. All tests were carried out with PRK cell cultures. Of all the compounds tested (Table 2), (E)-5-(2-bromovinyl)-dUrd and (E)-5-(2-iodovinyl)-dUrd emerged as the most potent anti-herpes agents: in order of decreasing activity; (E)-5-(2-bromovinyl)-dUrd > (E)-5-(2-iodovinyl)-dUrd > acycloguanosine ≈ araC > 5-bromo-dCrd > 5-iodo-dUrd > araT > 5-trifluoromethyl-dUrd > 5-ethyl-dUrd > 5-propyl-dUrd > 5-propynyloxy-dUrd > araA > phosphonoacetate > AIDDU > EHNA. For some of these compounds a similar order of activity has been established in a previous study (12): acycloguanosine > araC > 5-iodo-dUrd > trifluoromethyl-dUrd > araA > phosphonoacetate.

The minimum concentrations required for inhibition of cell growth were consistently higher than those required for inhibition of dUrd incorporation, and, in some instances, the minimum concentrations for cell growth inhibition could not be determined (Table 2). In general, the antiviral indexes, based on the ratio of the ID₅₀ for dUrd incorporation to the ID₅₀ for HSV-1 replication, showed the same order as the antiviral indexes based on the ratio of the ID₃₀ for total cell counts to the ID₅₀ for HSV-1 replication (Table 2). (E)-5-(2-Bromovinyl)-dUrd and (E)-5-(2-iodovinyl)-dUrd emerged as the most selective anti-herpes agents, followed by araT, 5-bromo-dCrd, 5-propyl-dUrd, and acycloguanosine. Somewhat less selective were 5-propynyloxy-dUrd and 5-ethyl-dUrd. Rather small selectivity indexes were recorded for 5-iodo-dUrd, phosphonoacetate, and araA, whereas araC, AIDDU, EHNA, and 5-trifluoromethyl-dUrd revealed little, if any, selectivity in their anti-herpes action.

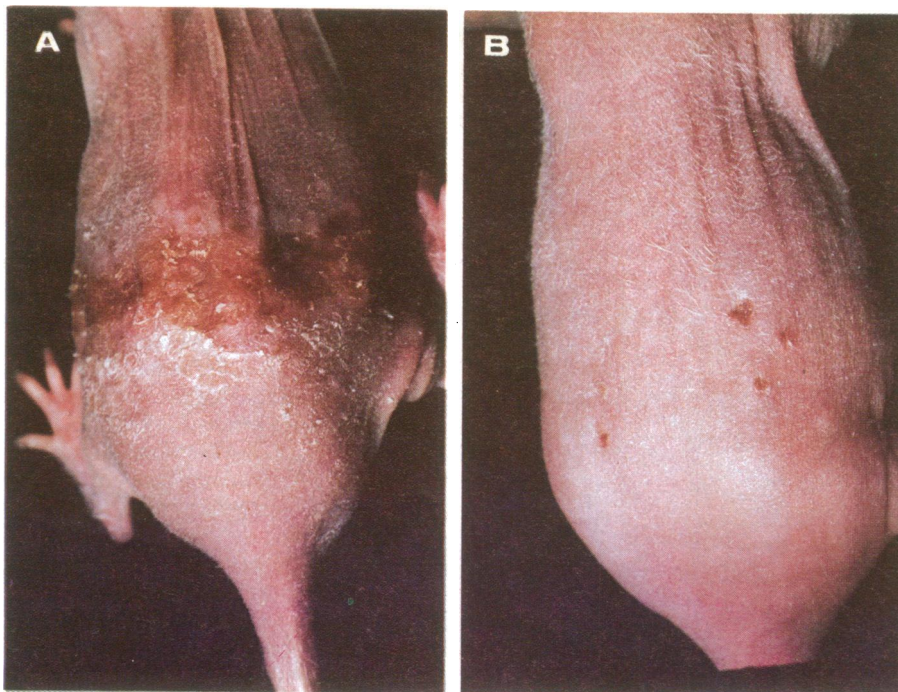


FIG. 3. Effect of topical administration of (*E*)-5-(2-bromovinyl)-dUrd on cutaneous herpes virus infection of athymic *nude* (*nu/nu*) mice. (A) Control mouse (treated with control ointment); (B) mouse treated with ointment containing 1% (*E*)-5-(2-bromovinyl)-dUrd. The pictures were taken 7 days after virus inoculation. Further details are described in the footnote to Table 3.

Treatment of cutaneous herpes virus infection

Next, we examined the efficacy of (*E*)-5-(2-bromovinyl)-dUrd in a cutaneous HSV-1 model infection of athymic *nude* (*nu/nu*) mice. This model is reminiscent of the experimental herpes skin infection of hairless mice (HRS/H strain) that has been used to evaluate the anti-herpes potentials of Kethoxal, 9- β -D-arabinofuranosyladenine, 5,6-dihydroxy-5-azathymidine, and various other compounds (17, 32–34). As described previously for hairless mice (33, 35), intracutaneous inoculation of *nu/nu* mice with HSV-1 (KOS) (on the midline of the spine) resulted in zoster-like skin lesions; the first symptoms to appear (usually on the fourth day after infection) were vesicles: these evolved to erosions which ulcerated and progressed downward from the site of inoculation to reach the midline of the abdomen by 6–8 days after inoculation (Fig. 3A). After the sixth day after infection, control mice started to show symptoms of neurologic involvement and paralysis, which ultimately resulted in death.

When (*E*)-5-(2-bromovinyl)-dUrd or 5-iodo-dUrd was applied topically (at 1% in a water-soluble ointment), only (*E*)-

5-(2-bromovinyl)-dUrd suppressed the development of cutaneous herpes lesions (Fig. 3B) and prolonged the life span of the mice (Table 3, Exp. 1). Under the same conditions, 5-iodo-dUrd neither inhibited lesion development nor prevented fatal outcome. The results presented in Table 3 (Exp. 1) were obtained with topical treatment of (*E*)-5-(2-bromovinyl)-dUrd initiated immediately after virus infection. If treatment was delayed until the fourth day, when lesions began to appear, topical application of (*E*)-5-(2-bromovinyl)-dUrd was no longer effective (data not shown). However, (*E*)-5-(2-bromovinyl)-dUrd proved effective in reducing the severity of the disease when administered intraperitoneally (at 1 mg/mouse; \approx 60 mg/kg) from the fourth to the seventh day after virus infection (Table 3, Exp. 2). This protective effect was most clearly demonstrated at the ninth day after infection, when all treated mice were still alive while 80% of the untreated mice had succumbed.

Preliminary toxicity experiments were carried out with the same mice as those used in the cutaneous herpes infection model. Twenty-five-day-old *nu/nu* mice (weighing 16–18 g) were given (*E*)-5-(2-bromovinyl)-dUrd (600 mg/kg) intra-

Table 3. Effect of (*E*)-5-(2-bromovinyl)-dUrd and 5-iodo-dUrd on the incidence of herpetic skin lesions and mortality of *nu/nu* mice inoculated intracutaneously with HSV

Compound		No. of mice with epidermal lesion*/total no. of mice alive										Mean survival time, days
Exp. 1	At day:	4	6	8	10	12	14	16	18	20		
	Control	0/10	7/10	8/8	4/4	3/3	2/2	2/2	1/1	—	9.5	
	5-Iodo-dUrd	0/10	5/10	9/10	5/5	1/1	1/1	1/1	1/1	1/1	10	
	(<i>E</i>)-5-(2-Bromovinyl)-dUrd	0/10	0/10	0/10	5/10	8/8	7/7	5/5	5/5	2/2	17	
Exp. 2	At day:	4	5	6	7	8	9	10	11	12		
	Control	0/10	3/10	6/10	9/9	6/6	2/2	1/1	—	—	8.2	
	5-Iodo-dUrd	0/10	0/10	4/10	6/8	6/6	5/5	4/4	2/2	—	9	
	(<i>E</i>)-5-(2-Bromovinyl)-dUrd	0/10	0/10	3/10	6/10	7/10	7/10	6/7	5/6	3/3	11.3	

Athymic *nude* (*nu/nu*) mice (25 days old), weighing 16–18 g, were inoculated intracutaneously (at the lumbar area) with HSV-1 (KOS) ($\approx 4.7 \log_{10}$ PFU per 0.05 ml per mouse) and treated *topically* twice daily for 6 days, starting immediately after virus infection, with a water-soluble ointment containing either no active ingredient or 1% 5-iodo-dUrd or 1% (*E*)-5-(2-bromovinyl)-dUrd (Exp. 1) or *systemically*, once daily from day 4 to day 7 after virus infection, with either 5-iodo-dUrd or (*E*)-5-(2-bromovinyl)-dUrd, both injected intraperitoneally at 1 mg per mouse (Exp. 2).

* Necrosis of at least 5–10 mm long.

peritoneally over an 8-hr period. These mice developed normally and at 5 days after injection of the drug their body weight had increased by 24%, as had their placebo-treated counterparts. Mice that had been treated with 5-iodo-dUrd, however, did not develop normally, and 5 days after intraperitoneal injection of the drug (600 mg/kg) their body weight had increased by only 4%.

Conclusions

As a potential anti-herpes drug, (*E*)-5-(2-bromovinyl)-dUrd offers several advantages over the standard antiviral compound 5-iodo-dUrd:

(i) In PRK and HSF cell cultures, (*E*)-5-(2-bromovinyl)-dUrd was about 20 times more active [against the replication of HSV-1 (KOS)] and 60 times less toxic (as monitored by dUrd incorporation into DNA) than 5-iodo-dUrd; in its activity against HSV-1 in PRK cells, (*E*)-5-(2-bromovinyl)-dUrd exceeded various other established anti-herpes compounds, including phosphonoacetic acid, araA, AIDDU, and acycloguanosine in both potency and selectivity.

(ii) In animal model systems—namely, cutaneous herpes infections in athymic *nude* mice—(*E*)-5-(2-bromovinyl)-dUrd suppressed the development of herpetic skin lesions and mortality therewith associated whether the drug was administered topically (as a 1% ointment) or systemically (at 60 mg/kg). The latter treatment was still efficacious when started at the time skin lesions (vesicles) had begun to develop (4 days after virus inoculation). When assayed under similar conditions, 5-iodo-dUrd did not markedly influence the course of the disease.

(iii) Blood drug levels obtained after intraperitoneal, subcutaneous, or oral administration of (*E*)-5-(2-bromovinyl)-dUrd to mice were significantly higher than those achieved with 5-iodo-dUrd. Active serum drug concentrations persisted for at least 5 hr if (*E*)-5-(2-bromovinyl)-dUrd was administered orally (unpublished data).

Whether (*E*)-5-(2-bromovinyl)-dUrd, like 5-iodo-dUrd and some other dUrd derivatives, is incorporated into viral or cellular DNA or both has not been determined. Unlike 5-iodo-dUrd, (*E*)-5-(2-bromovinyl)-dUrd does not activate the release of oncornavirus (RNA tumor virus) particles from nonproducer cell lines such as BALB/c mouse cells (unpublished data).

The mode of action of (*E*)-5-(2-bromovinyl)-dUrd and the basis for its selective anti-herpes activity remain to be established. Whatever target (*E*)-5-(2-bromovinyl)-dUrd may act upon, its extremely high anti-herpes index in cell culture combined with its efficacy and apparent freedom of toxicity in animal model systems warrant further evaluation of the drug in those experimental and clinical infections in which the use of 5-iodo-dUrd would normally be indicated.

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